FORM PTO-1390 (REV 11-98)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 1498-121
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) Unknown 09/786521
INTERNATIONAL APPLICATION NO. **PCT/GB99/02933**	INTERNATIONAL FILING DATE **3 September 1999**	PRIORITY DATE CLAIMED **7 September 1998**

TITLE OF INVENTION

METHOD FOR MONITORING THE TEMPERATURE OF A BIOCHEMICAL REACTION

APPLICANT(S) FOR DO/EO/US

LEE et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☒ has been transmitted by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☒ Amendments to the claims of the International Application under PCT Article 34.
   - a. ☒ are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. ☒ have been transmitted by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
   - d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (U.S.C. 371(c)(3)).

9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. To 16. Below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.

13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information. **PTO-1449/ International Search Report**

☐ This application is entitled to "Small entity" status. ☐ "Small entity" statement attached.

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
Unknown 09/786521	PCT/GB99/02933	1498-121

17. ☒ The following fees are submitted:

	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):	
-- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1000.00	
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00	
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2) paid to USPTO $710.00	
-- International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00	
-- International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).	$ 0.00

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE		
Total Claims	32	-20 =	12	X	$18.00	$ 216.00
Independent Claims	1	-3 =	0	X	$80.00	0.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)					$270.00	$ 270.00
					TOTAL OF ABOVE CALCULATIONS =	$ **1346.00**
Reduction by ½ for filing by small entity, if applicable. Small entity status must also be asserted. (Note 37 C.F.R. 1.9, 1.27, 1.28).						0.00
					SUBTOTAL =	$ **1346.00**
Processing fee of $130.00, for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)).					+	0.00
					TOTAL NATIONAL FEE =	$ **1346.00**
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). **$40.00** per property					+	$ 40.00
Fee for Petition to Revive Unintentionally Abandoned Application ($1240.00 – Small Entity = $620.00)						$ 0.00
					TOTAL FEES ENCLOSED =	$ **1386.00**
					Amount to be: refunded	$
					Charged	$

a. ☒ A check in the amount of $1386.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 14-1140 in the amount of $_____ to cover the above fees. A duplicate copy of this form is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed.
d. ☐ The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

NIXON & VANDERHYE P.C.
1100 North Glebe Road, 8th Floor
Arlington, Virginia 22201
Telephone: (703) 816-4000

SIGNATURE

Arthur R. Crawford
NAME

25,327 REGISTRATION NUMBER

March 6, 2001 Date

504579

METHOD FOR MONITORING THE TEMPERATURE OF A BIOCHEMICAL REACTION

The present invention relates to a method of carrying out an amplification reaction and in particular a polymerase chain reaction (PCR) using an internal temperature control mechanism.

A common problem in biochemical reactions, in particular miniaturised biochemical reactions is controlling the temperature. Invasive temperature probes add to the thermal mass of the sample and increase time constraints associated with heating and cooling. A particular example where such a problem occurs is with minaturised amplification reactions such as the PCR reaction. In this reaction, cycling between various accurate temperatures is an essential element. In outline, the procedure consists of the following steps, repeated cyclically. *Denaturation* : A mixture containing the PCR reagents (including the DNA to be copied, the individual nucleotide bases (A,T,G,C), suitable primers and polymerase enzyme) are heated to a predetermined temperature to separate the two strands of the target DNA.

Annealing : The mixture is then cooled to another predetermined temperature and the primers locate their complementary sequences on the DNA strands and bind to them.

Extension : The mixture is heated again to a further predetermined temperature. The polymerase enzyme (acting as a catalyst) joins the individual nucleotide bases to the end of the primer to form a new strand of DNA which is complementary to the sequence of the target DNA, the two strands being bound together.

Any interference with the reaching the predetermined temperatures as a result of the temperature measurement can present a significant problem in terms of the success of the amplification reaction.

The applicants have found a way in which the temperature present in a biochemical reaction can be monitored without the need for the application of temperature probes.

According to the present invention there is provided a method of monitoring the temperature of a biochemical reaction, said method comprising effecting the reaction in the presence of a fluorescently labelled temperature probe DNA sequence which comprises a double stranded region which denatures at a predetermined temperature, the fluorescent label or said temperature probe sequence being arranged so that the nature of the fluorescence changes at the point at which denaturation of the said region takes place; and monitoring fluorescence from said reaction mixture so as to determine when the said predetermined temperature has been reached.

The labelled temperature probe DNA sequence added to the reaction mixture in the method acts as a temperature probe allowing the temperature of the reaction to be accurately set without requiring external temperature probes.

The temperature probe DNA sequence may comprise a double stranded DNA sequence, or it may be in the form of a single nucleic acid strand, end regions of which hybridise together so as to form a loop or "hairpin" structure.

Suitable fluorescent labels include intercalating dyes, which are interposed between the strands of a double stranded region of a DNA sequence. When the double stranded DNA region containing the intercalating dye reaches the predetermined temperature, it will be denatured, thus releasing the intercalating dye present between the strands. At this point the fluorescence from the mixture will reduce significantly, giving a readable signal.

The process using a double stranded DNA sequence as a temperature probe is illustrated diagrammatically in Figure 1 hereinafter.

When intercalating dye (2) is added to a solution of double stranded DNA (1), it becomes interposed between the strands. The concentration of the dye (2) in this way produces a recognisable signal. On heating of the DNA so that it is denatured, dye is released and this event can be witnessed. Cooling to a temperature at which the said sequence will anneal again results in the intercalating dye becoming again trapped between the strands (see Figure 1).

Suitable intercalating dyes include SYBRGreen™, SYBRGold™ and ethidium bromide or other commercially available dyes.

Alternatively, the fluorescent label used in the method of the invention may utilise fluorescence resonance transfer (FRET) as the basis of the signal. These labels utilise the transfer of energy between a reporter and a quencher molecule. The reporter molecule is excited with a specific wavelength of light for which it will normally exhibit a fluorescence emission wavelength. The quencher molecule is also excited at this wavelength such that it can accept the emission energy of the reporter molecule by resonance transfer when they are in close proximity (e.g. on the same, or a neighbouring molecule). The basis of FRET detection is to monitor the changes at reporter and quencher emission wavelengths.

For use in the context of the present invention, the DNA sequence used as a temperature probe can be provided with a reporter and a quencher molecule, arranged so that the hybridisation of the strands alters the spatial relationship between the quencher and reporter molecules. Examples of such arrangements are illustrated in Figure 2 and Figure 3.

Figure 2 illustrates an Example where the temperature probe sequence is a single stranded "hairpin" type sequence (3), where the end portions hybridise together. A reporter molecule (4) is attached in the region of either the 5′ or the 3′ end of the sequence and a quencher molecule (5) is attached at the opposite end such that they are brought into close proximity

when the sequence is in the form of the loop. In this arrangement, FRET occurs and so fluorescent signal from the reporter molecule is reduced whilst the signal from the quencher (5) molecule is enhanced.

On denaturation however, the opposed end regions of the sequence separate so that the reporter and quencher molecules become spaced and so FRET no longer occurs. This changes the signals from the respective molecules and so this event can be detected.

Another arrangement is illustrated in Figure 3. In this case, the reporter (4) and quencher molecules (5) are located on different strands (6,7 respectively) of a DNA temperature probe sequence and are located such that on hybridisation of the strands, they are brought into close proximity to each other so that FRET can occur.

Yet a further embodiment is illustrated in Figure 4. In this case, an intercalating dye (2) is used as an element of the FRET system. A quencher molecule (5) which can absorb radiation from the dye may be arranged on a strand of the temperature probe sequence such that it can absorb radiation from dye which is close proximity to on hybridisation of the strands. When the temperature probe sequence reaches a temperature at which it is denatured, the dye (2) is dispersed and so the signal from the quencher molecule (5) changes.

This embodiment is advantageous in that only a single label need be applied to the temperature probe sequence. Single labelled sequences of this type are more economical to produce.

In yet a further embodiment (Figure 5), the reporter (4) and quencher (5) molecules are positioned on two oligonucleotide strands (9 and 10 respectively) which do not hybridise together. They are however designed so that in use, they hydridise to a DNA sequence present in the reaction mixture, which may be a plasmid (11), such that the reporter (4) and

quencher (5) are brought into close proximity and FRET can occur between them, giving a recognisable signal.

The DNA sequence to which they bind may be part of the reaction system, for example where the reaction being monitored is a PCR reaction wherein the DNA sequence comprises or is part of the amplification target sequence. Alternatively, the sequences may be added to the reaction in order to provide the basis for the temperature probe of the invention.

The temperature probe sequence of the invention may be designed so that it denatures at any desired predetermined temperature. For example, the denaturation temperature of a sequence depends to some extent on its length. Longer sequences will denature or melt at higher temperatures. Furthermore, it is known that the bases C and G bind together more strongly than A and T. Therefore, the greater the higher the content of the bases G and C contained within a sequence, the higher the melting point of the sequence will be. This feature is illustrated in Figure 5 which shows the melting temperature of a DNA sequence plotted against the percentage of and GC base pairs which are present within in. Thus, by adjusting the GC content, the temperature probe sequence may be designed so that, if desired, it also has a predetermined length.

The method of the invention is particularly applicable for use in amplification reactions such as the polymerase chain reaction (PCR). In this case, the temperature probe sequence of the invention is introduced into the reaction vessel. Suitably the temperature probe sequence is designed such that it generates a detectable signal when it reaches the optimum annealing temperature of the target DNA sequence as this is intermediate temperature is most difficult to set accurately in practice. However, more than one such temperature probe sequence may be added and arranged to provide appropriate and preferably different signals when the predetermined extension and/or denaturation temperatures have been reached.

The invention will now be particularly described by way of example with reference to the accompanying diagrammatic drawings in which:

Figure 1 illustrates the formation and use of a labelled temperature probe sequence for use in the method of the invention;

Figures 2 to 5 represent alternative embodiments of the labelled temperature probe sequences of the invention and the denaturation thereof;

Figure 6 illustrates a construct used in the examples hereinafter; and

Figure 7 shows the melting temperature of plasmid constructs and inserts as measured using the method of the invention, as a function of the percentage GC content of the construct, where the lighter line represents the oligonucleotides and the darker line represents the -47/-48 amplicon from constructs.

Example 1

Oligonucleotides, 60 base pairs in length, were designed by randomly removing the letters G, A, T and C from a paper bag. Complementary pairs of the thus formed random oligonucleotides were mixed together at a final concentration of 1μM and 1:40,000 dilution of SYBRGreen™ reference dye. The mixtures were then loaded into LightCycler™ tubes and the temperature slowly raised from 40°C to 110°C. The fluorescence at 520nm was measured and was seen to drop off as the temperature was raised. The differential of fluorescence was used to determine the peak rate of change (i.e. drop) which corresponds to the strands melting. 20%, 40%, 50% 60% and 80% GC oligos were used in different experiments. The results, expressed as a graph of melting temperature vs GC content is shown as Figure 7.

Example 2

The different GC duplexes used in Example 1 were cloned into the vector polylinker of pUC19 plasmid as illustrated in Figure 6. This plasmid was subjected to a polymerase chain reaction using vector primer sites, the -47 and -48 sequencing primer sites. The PCR reaction contained 1:40,000 dilution of SYBRGold™ reference dye. After PCR on the LightCycler™, the products were melted off as described in Example 1. The melting temperature of the different amplicons vs the GC content is shown on the graph (Figure 7).

Claims

1. A method of monitoring the temperature of a biochemical reaction, said method comprising effecting the reaction in the presence of a fluorescently labelled temperature probe DNA sequence which comprises a double stranded region which denatures at a predetermined temperature, the fluorescent label of said temperature probe sequence being arranged so that a detectable signal occurs at the point at which denaturation of the said region takes place; and monitoring fluorescence from said reaction mixture so as to determine when the said predetermined temperature has been reached.

2. A method according to claim 1 wherein the temperature probe DNA sequence comprises a labelled double stranded DNA sequence.

3. A method according to claim 1 wherein the temperature probe DNA sequence comprises a single nucleic acid strand, end regions of which hybridise together so as to form a loop or "hairpin" structure.

4. A method according to any one of the preceding claims wherein the fluorescent label comprises an intercalating dye.

5. A method according to claim 4 wherein the intercalating dye comprises SYBRGreen™ or SYBRGold™ or ethidium bromide.

6. A method according to any one of claims 1 to 5 wherein the fluorescent label used in the method of the invention may utilise fluorescence resonance transfer (FRET) as the basis of the signal.

7. A method according to claim 6 wherein the temperature probe DNA sequence is provided with a reporter and a quencher molecule, arranged so that the hybridisation of the strands

alters the spatial relationship between the quencher and reporter molecules.

8. A method according to claim 7 wherein the temperature probe sequence is a single stranded sequence, where the end portions hybridise together and wherein the reporter molecule is attached in the region of either the 5′ or the 3′ end of the sequence and the quencher molecule is attached at the opposite end.

9. A method according to claim 8 wherein the reporter and quencher molecules are located on different strands of a DNA temperature probe sequence such that on hybridisation of the strands, they are brought into close proximity to each other.

10. A method according to claim 9 wherein FRET is established between an intercalating dye and a quencher molecule arranged on a strand of the temperature probe sequence such that it can absorb radiation from dye which is in close proximity on hybridisation of the strands.

11. A method according to claim 7 wherein the temperature probe DNA sequences comprises a first DNA strand having a reporter molecule thereon, a second DNA strand having a quencher molecule thereon, said first and second DNA strands being designed to hybridise to a third DNA strand such that the reporter and quencher molecules are brought into close proximity with each other.

12. A method according to any one of the preceding claims wherein the length of the temperature probe sequence is used to set the said predetermined temperature.

13. A method according to any one of the preceding claims wherein the GC content of the temperature probe sequence is modified to obtain the desired predetermined temperature.

14. A method according to any one of the preceding claims wherein the biochemical reaction is an amplification reaction.

15. A method according to claim 14 wherein the amplification reaction is a polymerase chain reaction (PCR).

16. A method according to claim 15 wherein the length of the temperature probe sequence is similar to that of an amplicon of the PCR reaction.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/68

A1

(11) International Publication Number: **WO 00/14278**

(43) International Publication Date: 16 March 2000 (16.03.00)

(21) International Application Number: PCT/GB99/02933

(22) International Filing Date: 3 September 1999 (03.09.99)

(30) Priority Data:
9819417.8 7 September 1998 (07.09.98) GB

(71) Applicant *(for all designated States except US)*: THE SECRETARY OF STATE FOR DEFENCE [GB/GB]; Defence Evaluation and Research Agency, Ively Road, Farnborough, Hampshire GU14 0LX (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LEE, Martin, Alan [GB/GB]; CBD, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). BRIGHTWELL, Gale [GB/GB]; CBD, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB).

(74) Agent: BOWDERY, A., O.; D/IPR, Formalities Section, Poplar 2, MOD Abbey Wood #19, Bristol BS34 8JH (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR MONITORING THE TEMPERATURE OF A BIOCHEMICAL REACTION

(57) Abstract

A method of monitoring the temperature of a biochemical reaction such as an amplification reaction is described. The method comprises effecting the reaction in the presence of a fluorescently labelled temperature probe DNA sequence which comprises a double stranded region which denatures at a predetermined temperature, the fluorescent label of said temperature probe sequence being arranged so that a detectable signal occurs at the point at which denaturation of the said region takes place; and monitoring fluorescence from said reaction mixture so as to determine when the said predetermined temperature has been reached.

Fig.1.

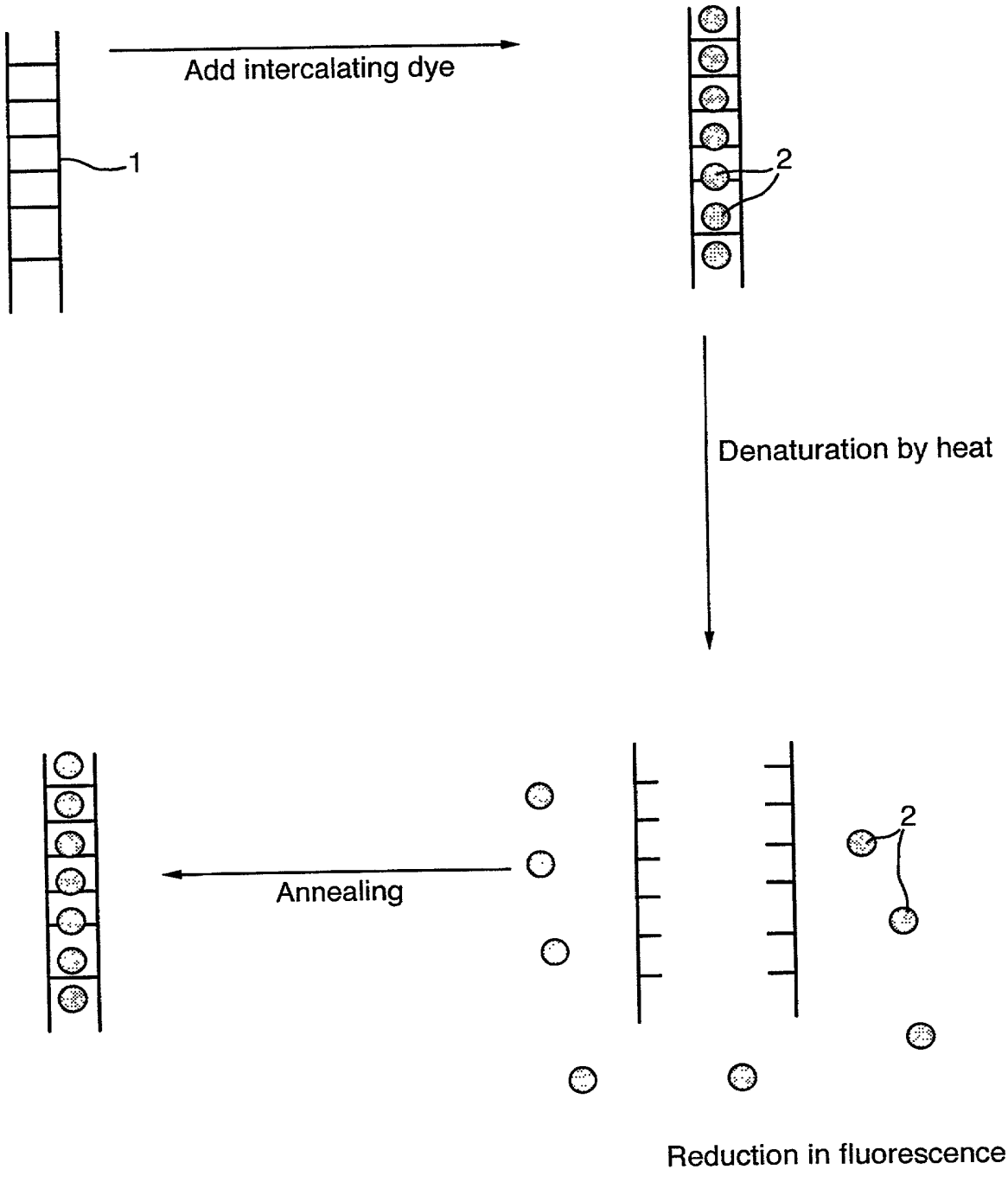

Add intercalating dye
1
2
Denaturation by heat
Annealing
2
Reduction in fluorescence

Fig.2.

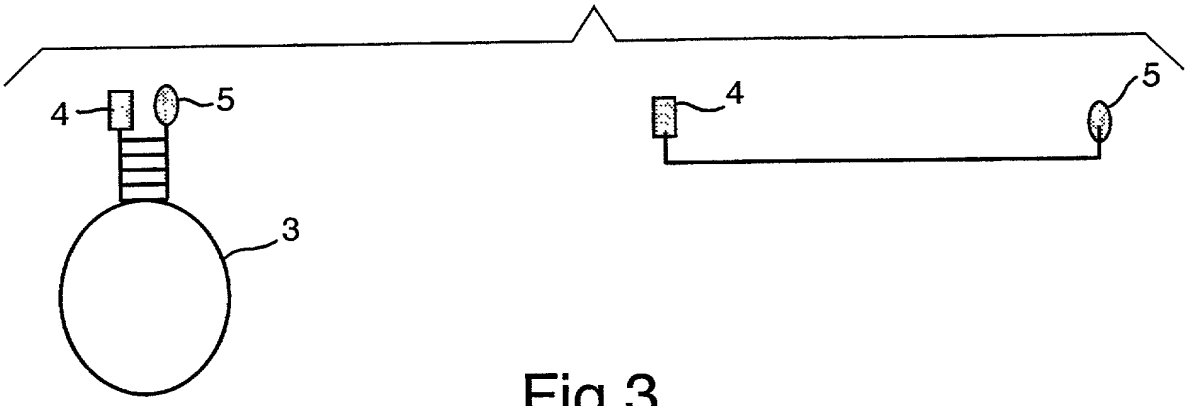


Fig.3.

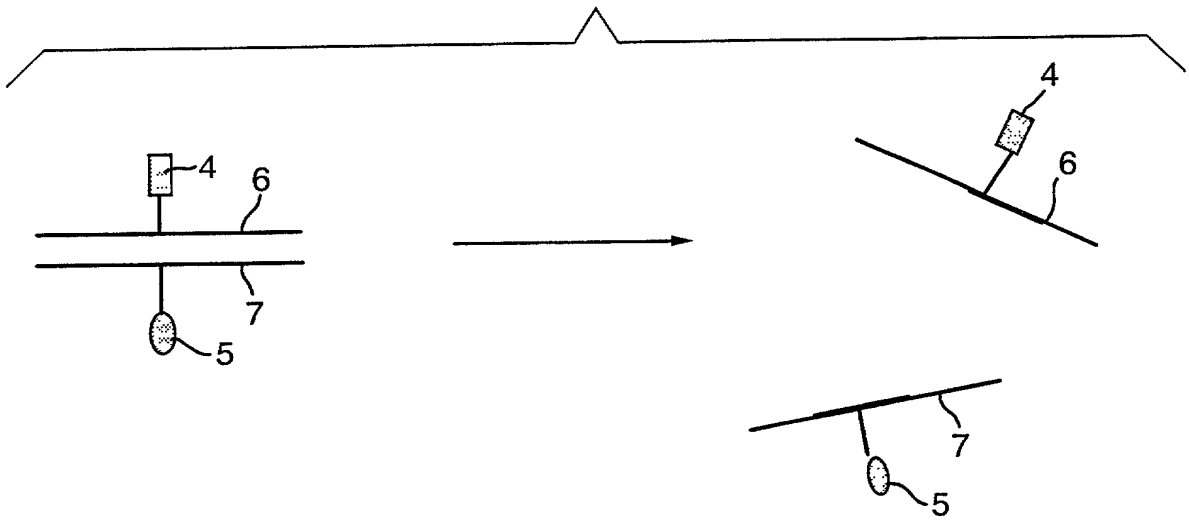


Fig.4.

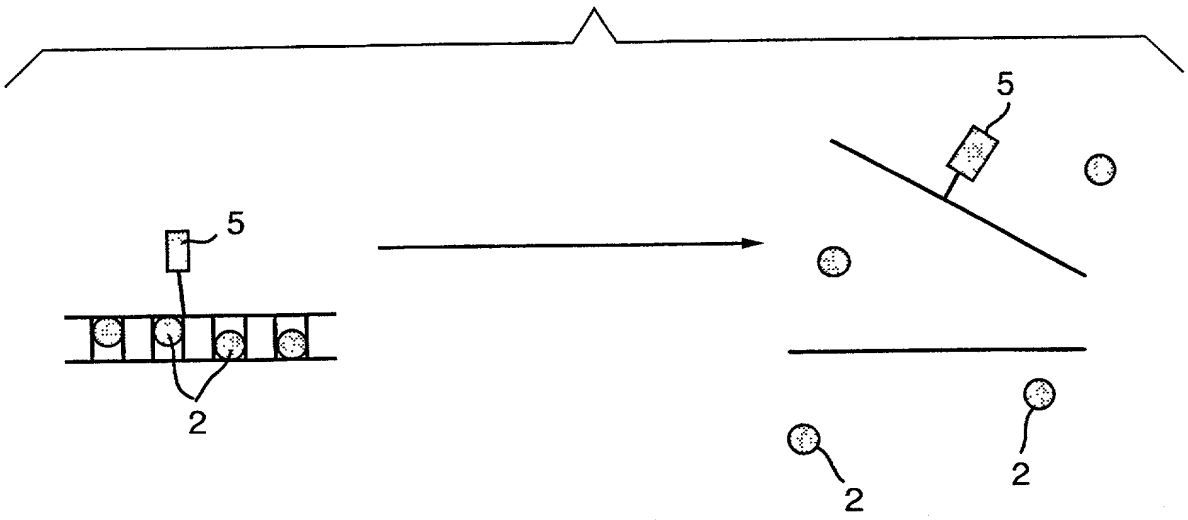


Fig.5.

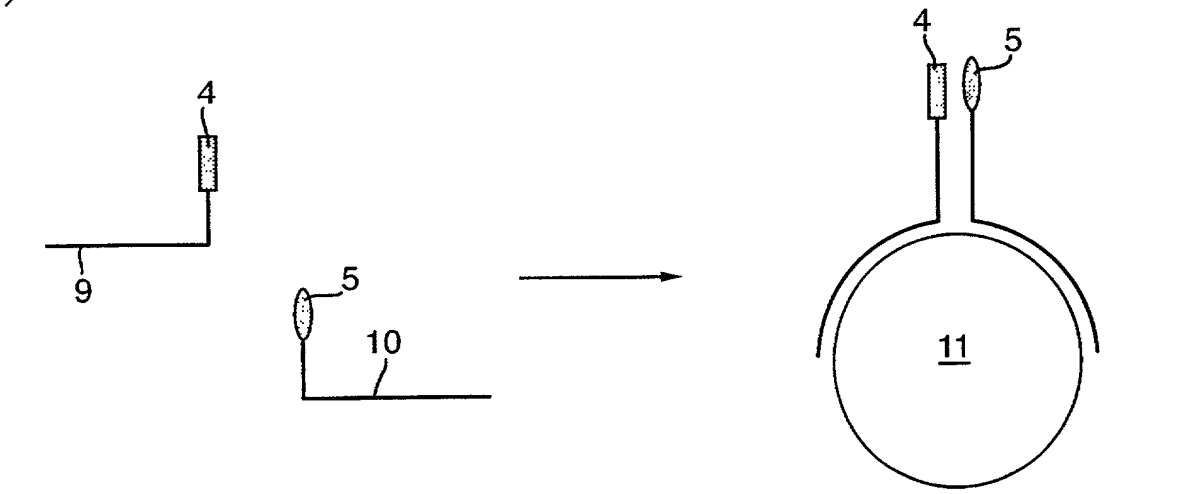
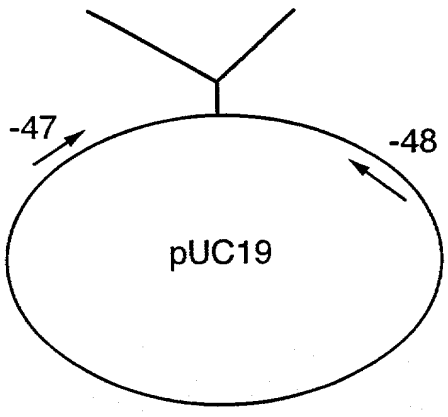


Fig.6.

INTERNAL CONTROLS

20%
40%
50%
60% or
80% GC



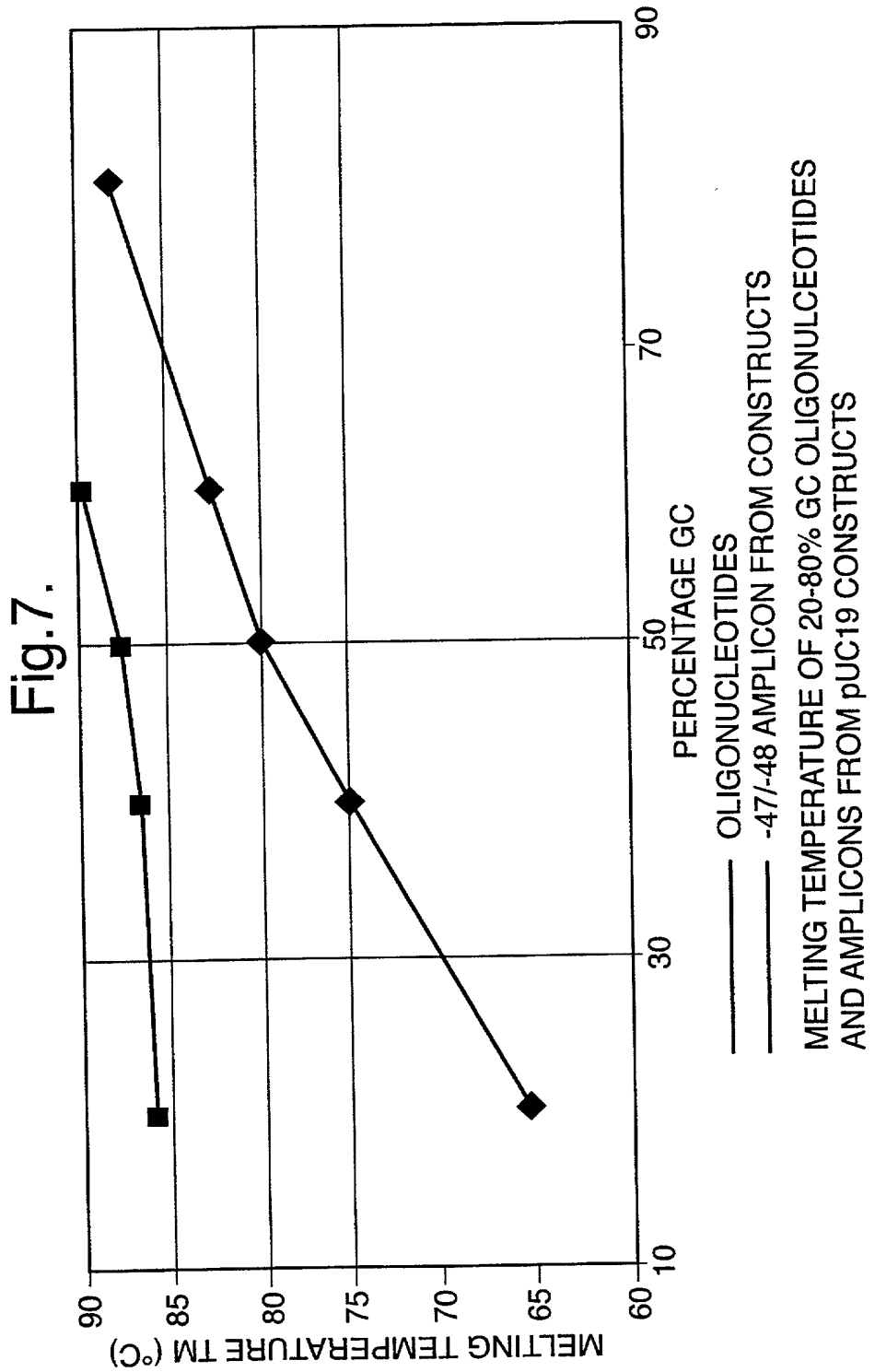

MELTING TEMPERATURE TM (°C)
90
85
80
75
70
65
60
10
30
50
70
90
PERCENTAGE GC
OLIGONUCLEOTIDES
-47/-48 AMPLICON FROM CONSTRUCTS
MELTING TEMPERATURE OF 20-80% GC OLIGONULCEOTIDES
AND AMPLICONS FROM pUC19 CONSTRUCTS

Fig.7.

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As below named inventor, I hereby declare that my residence. Post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

METHOD FOR MONITORING THE TEMPERATURE OF A BIOCHEMICAL REACTION

The specification of which (check applicable box(s)):

[ ] is attached hereto.

[ ] was filed on _____

as U.S. Application Serial No. _____

[X] was filed as PCT international application No. PCT/GB99/02933 filed 3 September 1999

and (if applicable to U.S. or PCT Application) was amended on

4 September 2000

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 C.F.R. 1.56(A). I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s): Application Number	**County**	**Day/Month/Year Filed**
9819417.8	**GB**	**7 September 1998**

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT international applications listed above and below, insofar as the subject matter of each of the claims of this application is not disclosed in such prior applications in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. 1.56(a) which occurred between the filing date of the prior applications and the national or PCT international filing date of this application:

Prior U.S. /PCT Applications(s):	**Day/Month/Year Filed**	**Status:**
PCT/GB99/02933	**3 September 1999**	**Pending**

I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issued thereon. And I hereby appoint **NIXON & VANDERHYE P.C. 8th Floor, 1100 North Glebe Road, Arlington, Virginia 22201-4714 Telephone number (703) 816-4000 to who all communications are to be directed).** And the following attorneys thereof (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Arthur R. Crawford, 25327; Larry S. Nixon 25640; Robert A. Vanderhye, 27076; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C Mitchard, 29009; Duane M Byers, 33363; Paul J. Henon, 33626; Jeffry H. Nelson, 30481; John R. Lustova, 33149; H. Warren Burnam, Jr., 29366; Thomas E. Byrne, 32205; Mary J Wilson 32955; J Scott Davidson 33489

Inventors Signature _____ Date 31/01/01

Inventors Name (typed)

Martin	A	LEE	GB
First	Middle Initial	**Family name**	Citizenship

Residence (city) Salisbury (State/Foreign Country) GB GBN

Post Office Address CBD Porton Down, Zip Code SP4 0JQ

Salisbury, Wiltshire.

Inventors Signature _____ Date 8-2-1

Inventors Name (typed)

Gale		BRIGHTWELL	GB
First	Middle Initial	**Family Name**	Citizenship

Residence (City) Salisbury State/Foreign Country) GB GBN

Post Office Address, CBD Porton Down Zip Code SP4 0JQ

Salisbury, Wiltshire.